THE IDENTITY OF *ENTAMEBA HISTOLYTICA* AND *ENTAMEBA TETRAGENA*, WITH OBSERVATIONS UPON THE MORPHOLOGY AND LIFE CYCLE OF *ENTAMEBA HISTOLYTICA*.*

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(WITH PLATES 1 AND 2.)

During the past three years a great deal of study has been devoted to the parasitic amebae of man, especially to the species that have been described as causing dysentery, and considerable advance has been made in our knowledge of these organisms. Among the subjects investigated the possible identity of *Entameba histolytica* and *Entameba tetragena*, the species most often described as associated with amebic dysentery, has attracted much attention, and the impression has been steadily gaining ground that these organisms, generally considered as being distinct species, are in reality identical.

Until quite recently I have considered *E. histolytica* and *E. tetragena* as distinct species, but the study of certain material, to be hereafter described, has convinced me that *E. tetragena* is identical with *E. histolytica;* that Schaudinn and, afterward, myself, incorrectly interpreted certain changes occurring in this entameba as reproduction by "budding" or spore formation; and that to Viereck, whose description was amplified by Hartmann, belongs the credit of first correctly describing the life cycle of *E. histolytica*, although he named the entameba which he described as a new species, *E. tetragena*.

In order thoroughly to understand the exact position of the question of the identity of these entamebae at the present time, it is necessary to review the literature dealing with this particular point *in extenso*, and in doing so I shall quote at length from the more important papers, in order that each investigator's position in the matter may be clearly understood.

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In 1903, Schaudinn[1] published a paper upon the amebae of the intestinal tract of man, in which he accepted Casagrandi's and Barbagallo's division of amebae into two genera, *Ameba* and *Entameba*, the latter genus including all of the parasitic species, and demonstrated that in the intestine of man there occur two distinct species, one common to healthy individuals and those suffering from diseases other than dysentery, while the other was found only in patients suffering from amebic dysentery. To the former he gave the name *Entameba coli*, and to the latter, the name *Entameba histolytica*. He based his differentiation of these species upon marked differences in morphology and in their life cycle.

Schaudinn described *E. coli* as an entameba showing but little distinction between the ecto- and endoplasm; having a distinct nucleus possessing a rather thick nuclear membrane and a well marked karyosome; reproducing by simple division; schizogony, with the formation of eight amebulae, and by the formation of cysts containing eight daughter nuclei.

He described *E. histolytica* as an entameba showing a marked distinction between the ecto- and endoplasm; a nucleus characterized by a delicate nuclear membrane and a minute karyosome, the nucleus being generally invisible in the living organism; and as reproducing by simple division and by a process of "budding" or spore formation. The latter process he described as consisting of the budding off, from the periphery of the organism, of minute portions of cytoplasm containing nuclear chromatin, the buds later developing a resistant membrane, and only undergoing further development when they reached the intestinal canal of a suitable host. Schaudinn states that he produced typical dysentery in two cats by feeding them material containing these spores and that typical entamebae appeared in the feces of the experimental animals.

I was able to confirm Schaudinn's observations in 1905[2] and my results will be referred to later in this communication.

Viereck,[3] in 1906, described what he considered to be a new species of entameba in a patient suffering from dysentery contracted in Africa, and called it *E. tetragena*. The same parasite was described almost simultaneously by Hartmann,[4] and since then this species has been found to have a worldwide distribution and the observations of these authorities regarding it have been confirmed by many investigators.

E. tetragena was described by Viereck and Hartmann as an entameba showing a marked distinction between the ecto- and endoplasm; having a distinct nucleus possessing a rather thick nuclear membrane; a large karyosome containing generally a distinct centriole; while it reproduced by simple division and by the formation of cysts containing four daughter nuclei, thus distinguishing it, according to these authors, from *E. coli*, which forms cysts containing eight daughter nuclei, and from *E. histolytica*, which was said to reproduce by "budding" or spore formation.

In 1909, Hartmann[5] published a monograph upon *E. histolytica* in which he confirmed Schaudinn's description of this species and pictured forms which he interpreted as "budding forms," or reproduction by spore formation. In this publication he says: "Since the publication of Schaudinn, *Entameba histolytica* has been accurately described only by Craig, Werner, and myself, and Schaudinn's description thereby confirmed."

[1] *Arb. a.d.k. Gsndhtsamte.*, 1903, 19, p. 547.

[2] *Am. Med.*, 1905, 9, pp. 854, 897, and 937.

[3] *Arch. f. Schiffs- u. Tropen.-Hyg.*, 1907, 11, p. 1.

[4] *Ibid.*, 1908, 5, p. 117.

[5] *Arch. f. Protistenk.*, 1909, 18, p. 207.

It is thus evident that at this time Hartmann was convinced that *E. histolytica* was a distinct species and that the description given by Schaudinn had been confirmed by himself, as well as others.

In Hartmann's excellent chapter entitled "Die Dysenterie-Amöben" in S. von Prowazek's *Handbuch der Pathogenen Protozoen* (1911), he casts doubt upon the accuracy of his former description of *E. histolytica* and states that he evidently mistook degenerative forms of *E. tetragena* for certain stages in the life cycle of *E. histolytica*, and that, after studying very carefully all of Schaudinn's material, he has come to the conclusion that in only one case, an infection contracted in China, can he confirm the description of *E. histolytica* as given by Schaudinn. He calls attention to the probability that many of the so-called "budding" forms were really degenerative in character and that similar forms occur in infections with *E. tetragena*.

In a still later monograph upon *E. tetragena*, published in 1912,[1] Hartmann describes in detail the character of the nucleus in this species, a structure he considers of the greatest importance in differentiating this species from other entamebae, and states that he considers *E. histolytica* as a rather doubtful species. He believes thus, because *E. tetragena* is the only entameba he has encountered in a considerable number of cases, and because of his failure to find any evidence of reproduction by "budding" or spore formation in this dysentery entameba.

At the present writing, Hartmann's position is apparently as follows: He does not absolutely deny the existence of *E. histolytica* as a distinct species but inclines strongly to the belief that it is identical with *E. tetragena*.

In 1911, Major Whitmore, U.S. Army Medical Corps, published a paper[2] detailing the results of his study in Hartmann's laboratory of material from cases of amebic dysentery contracted in the Philippine Islands and Saigon. He confirms the presence in Manila of *E. coli*, first noted by myself in soldiers returning from the Philippines, but finds that the entamebae present in all of his material from dysenteric cases was *E. tetragena*, not a single case of infection with *E. histolytica* being observed. He therefore concludes that the common dysentery entameba of Manila is *E. tetragena* and that infections with *E. histolytica* must be very uncommon.

In a later paper[3] Whitmore accepts the specific status of *E. histolytica* and *E. tetragena* but, in speaking of the encystment of the latter species he says:

"Under the conditions that cause *tetragena* to encyst, there are always a great many degenerating amebae in which the nucleus is in all stages of disintegration, and great care is necessary in order not to misinterpret such findings. Again, at this time, the chromatin is passing off from the nucleus into the plasma to form chromidia, and such an appearance may easily be mistaken for the nuclear changes which take place in *histolytica* preparatory to budding."

Major Whitmore states[4] that he believes *E. tetragena* and *E. histolytica* to be identical and that it would appear that the name *tetragena* should be abandoned in favor of *histolytica*.

[1] *Arch. f. Protistenk.*, 1912, 24, p. 163.
[2] *Ibid.*, 1911, 23, p. 70.
[3] *Arch. Int. Med.*, 1912, 4, p. 515.
[4] Personal communication.

The identity of these two species was discussed very fully in a paper by Walker,[1] who studied the subject in Manila. This observer was the first definitely to state that *E. tetragena* is identical with *E. histolytica*, and his description of the morphology and life cycle of the latter parasite I believe to be more nearly correct than that given by any other investigator. Practically all that has been accomplished since the publication of his paper has been confirmatory of his work and at the present time I believe that his conception of the specific status of *E. histolytica* and *E. tetragena* is the correct one. Walker's paper is of especial interest because he dealt with a large amount of material in Manila where infection with entamebae is so frequently observed.

After discussing the amebae found in the water supply of Manila and those which can be cultivated, he gives his results in the study of the amebae found in the intestine of man. He concludes that all of the amebae which can be cultivated are free living species, belonging to the genus *Ameba*—a conclusion previously reached by the writer and confirmed by Hartmann, Whitmore, and others—and that the amebae found in the intestine of man are strictly parasitic and belong to the genus *Entameba*, as shown by the same investigators. He also confirms the presence of the harmless entameba, *E. coli*, in Manila, but recognizes only one species of pathogenic entameba, i.e., *E. histolytica*, which he thinks includes the entameba described by Viereck and Hartmann as *E. tetragena*, and that described by Elmassian as *E. minuta*. He says, after describing *E. coli:* "The other species includes the *histolytica* variety of Schaudinn, the *tetragena* variety of Viereck and Hartmann, and probably the *minuta* variety of Elmassian. It is characterized by the hyaline appearance, indistinct nucleus, and active motility in the living entameba; by the feebly staining, reticulated cytoplasm, and by the relative paucity of chromatin which is arranged either as a barely perceptible layer about the inner surface of the nuclear membrane, with or without a few fragments scattered in the nuclear network (*histolytica* variety), or as a more extensive but loose, granular peripheral layer and a loose central karyosome (*tetragena* variety), with transitions between these two varieties in the stained entamebae; and especially by the development of cysts containing four nuclei. This species is found only in the stools, pus, or tissues of amebic dysentery, amebic abscesses, or of cases having a history of amebic dysentery, and is probably a pathogenic species. According to the law of priority this species should bear the name *Entamoeba histolytica* Schaudinn."

Walker is thus the first to recognize that the nuclear structure of *E. histolytica* varies greatly under different conditions, at times presenting the typical *histolytica* type of structure, described by Schaudinn and myself; while at others it presents the typical *tetragena* type, described by Viereck and Hartmann.

In his paper two cases are described in which, during the active stage of dysentery, the entamebae were all of the *histolytica* type, and no cysts could be found, but when the symptoms disappeared and the stools became normal, the characteristic *tetragena* cysts appeared. He rightly says:

"During the active phase of amebic dysentery only the trophozoites of these entamebae are present in the stools. . . . . When the acute symptoms in untreated

[1] *Philippine Jour. Sc.*, 1911, 6, p. 379.

dysentery have passed and the stools of the patient are becoming normal, the trophozoites become smaller, less actively motile, and more rounded in the resting forms, and the chromatin becomes more abundant in the nucleus. These changes are preparatory to the development of cysts. . . . . Finally, encysted forms containing four nuclei appear which may persist for an indefinite period or until the patient suffers from an exacerbation of the acute symptoms."

Walker was unable to find any forms which he could interpret as showing reproduction by "budding" or spore formation. His description of the two types of nuclei observed in *E. histolytica* is so accurate that it is here reproduced:

"Two varieties in the distribution of the chromatin can be distinguished. In the one the chromatin is arranged as a barely perceptible layer about the inner surface of the nuclear membrane, with or without a minute karyosome, or a few scattered fragments in the nuclear network. This variety corresponds with the *histolytica* species of Schaudinn. In the second variety the chromatin is rather more abundant and is arranged in part as a loose granular layer, that frequently shows radial projections, about the inner surface of the nuclear membrane; and in part as a loose central karyosome which, in its most typical form, consists of a minute centriole surrounded by an achromatic halo that is bounded by a circle of chromatic granules. This variety corresponds with the *tetragena* species of Viereck and Hartmann."

Walker found that entamebae showing the *histolytica* type of nucleus were observed more frequently in Manila than those showing the *tetragena* type, thus confirming my own observations in this respect.

He concludes his valuable paper by stating that he recognizes two species of entamebae as being present in his material: a harmless species, *E. coli*, and a presumably pathogenic species, *E. histolytica*, which includes the species described by Viereck and Hartmann, and named *E. tetragena;* and that it is possible to differentiate these species by means of the microscope. His paper is accompanied by photomicrographs illustrating the variations in the type of nucleus observed in *E. histolytica.*

Several valuable papers, published by Darling upon the entamebae observed in the Canal Zone, will now be considered.

In a report[1] of the Board of Health Laboratory, Canal Zone, published in 1912, he states that investigations demonstrate that *E. histolytica* "is the chief, if not the sole, pathogenic entameba in this region."

Regarding *E. tetragena* he notes that this species has been found twice and says: "This species of entameba is not frequently encountered and it is not regarded as being a very pathogenic type."

[1] *Report of Board of Health Lab., Dept. Sanitation, Isthmian Canal Commission,* 1912, p. 42.

As showing how liable one is to err if one depends largely upon nuclear structure in the differentiation of species of entamebae, Darling's results are of interest and value, for in this paper he concludes from the study of the structure of the nucleus that the common entameba of Panama is *E. histolytica*. He says:

"In order to determine positively, if possible, the type of entameba that has actually invaded the tissues of man in this region, a variety of pathological material has been examined with regard to this point, paying particular attention to the morphology of the nucleus of the invading entameba as stained by hematoxylin, there being structural differences between the nuclei of *E. histolytica*, *E. tetragena*, and *E. coli* when stained carefully with hematoxylin. In only one instance out of 22 individuals that had died of entamebic dysentery was an entameba found in the tissues resembling *E. tetragena*—though not positively identified as such. In all other instances the entamebae correspond with *E. histolytica*."

The author closes this contribution by stating that "*E. histolytica* is the chief, if not the sole, pathogenic entameba in this region," but in a paper published about six months later[1] Darling reverses his conclusions regarding *E. histolytica* and says:

"From a careful study of entamebae obtained from clinical cases and autopsy material in this region, I am not only of the opinion that the only pathogenic entameba of Panama is *E. tetragena*, but I am in agreement with Hartmann and Doflein that the entameba, usually described as *E. histolytica* from cases of dysentery, is *E. tetragena*."

Darling bases this statement upon the occurrence of cysts containing four nuclei in his material, the absence of budding forms described by Schaudinn and myself, and the fact that, in infected kittens, vegetative forms were observed that showed the *histolytica* type of nucleus:

"Many trophozoites were found which had the staining and morphological characters not only of *E. tetragena*, but of *E. histolytica* and *E. nipponensis*. These variations in the morphology of the nucleus depend upon variable amounts of chromatin and variations in the distribution of the same within the nuclear membrane." Regarding the occurrence of cysts in his cases, he says: "The likelihood of finding cysts in any given case depends on several circumstances. In progressively fatal cases in man and animals in which the individual succumbs to infection, there may at times be seen, in addition to the large trophozoites, some small forms containing idiochromidia, but I have never detected cysts in these cases. In early acute cases, in first infections, for example, where the lesions were presumably superficial, and where active medication by mouth had been administered, I failed to find developmental forms."

In January, 1913, Darling, in another paper,[2] definitely states his belief that *E. tetragena* is the only pathogenic entameba:

[1] *Jour. Trop. Med.*, 1912, 15, p. 257.

[2] *Arch. Int. Med.*, 1913, 11, p. 1.

"If we make a careful study of the entamebae in our cases of dysentery and liver abscess, using a technic which brings out, as well as possible, the morphological features of the nucleus of trophozoites and in the cysts, we can be of no other opinion than that there is but one pathogenic entameba and that one is *E. tetragena*." In this paper Darling states that treatment appears to have a great influence in preventing the development of cysts, and, as will be seen later, this undoubtedly explains why cysts have not been observed in many infections with *E. histolytica*.

In still later contributions[1] Darling describes the occurrence, in kittens infected with *E. tetragena*, of forms identical with *E. histolytica*, especially the forms answering to the description of "budding" or spore formation, as given by Schaudinn and others. His description of these forms is as follows:[2]

"The material showing the budding forms was in the fourth remove, at the time of the death of the kittens, that is, when the strain had become mature or senile, reduced in size until it resembled *E. minuta*, when chromidia had appeared in every trophozoite, and a few cysts had appeared. In well fixed hematoxylin preparations the peculiar changes noted in some of the trophozoites were as follows:

"1. The appearance of buds of different sizes on the periphery of the trophozoites in question. These buds were usually clear and free from chromidia. Some, however, contained chromidia and not infrequently a bud contained the nucleus.

"2. The extrusion of chromidia, nuclei, and portions of cytoplasm. Chromidia was seen apparently being extruded. The nucleus always took up an extreme peripheral position in the cytoplasm, was frequently attached by a slim pedicle of cytoplasm and later detached. Round portions of the cytoplasm [buds] were extruded."

Darling regards these forms as degenerative in character and believes that his observations "establish a correlation between the findings of Schaudinn and Craig on the one hand, and those of Viereck, Werner, and Hartmann on the other."

It will be noted that Darling found these "budding" forms in wet-fixed, hematoxylin-stained preparations, thus confirming my own results and disproving the statement of some authorities that such appearances are found only in preparation air-dried and stained with some modification of the Romanowsky stain, and that they were due to the staining or fixing process.

At the present writing Darling evidently believes that *E. tetragena* is the only pathogenic entameba and that *E. histolytica* was described from degenerative forms of *tetragena*. While his observations are largely confirmatory of those of Walker, already noted, he has thrown considerable light upon the occurrence of the

[1] *Science*, 1913, 26, p. 16; *Jour. Am. Med. Assn.*, 1913, 60, p. 1220.

[2] *Op. cit.*

so-called "budding" and has shown that such forms occur only under certain conditions.

In a valuable paper, published in 1912, Wenyon[1] gives the results of experimental research upon the production of dysentery in cats with *E. histolytica* and *E. coli*. Regarding the identity of *histolytica* and *tetragena* he says:

"It seems highly probable that the pathogenic ameba with which Schaudinn worked was the common pathogenic form and that he failed to recognize the cysts of Viereck with their four nuclei and that the small spores he described were other structures unconnected with the amebae. If this position be adopted it comes about that the ameba of Viereck, *E. tetragena*, is none other than *E. histolytica* of Schaudinn, and that Viereck regarded it as a distinct species because he had discovered the true life history, which, of course, differed from the erroneous one given by Schaudinn. Hence, provided Schaudinn was right in naming the pathogenic ameba *E. histolytica*, the name *E. tetragena* is no longer required. The common pathogenic ameba of man is still *E. histolytica*, but the life history is not that described by Schaudinn, but that discovered by Viereck."

Wenyon calls attention to the fact that the characteristic cysts occur only in a small proportion of cases of amebic dysentery, and that he has not been able to confirm the occurrence of reproduction by "budding" in *E. histolytica*. Wenyon was successful in producing dysentery and liver abscess in cats with this entameba but all of his experiments with *E. coli*, the harmless entameba, resulted negatively. His successful case of liver infection in a kitten was long antedated by that of the writer, who produced liver abscess in a kitten with *E. histolytica* as far back as 1905.[2]

The latest contribution upon the subject is that of James, who has studied a large amount of material upon the Isthmus of Panama and in the Canal Zone. I have had the pleasure of examining many of his preparations. In a paper published in April, 1913,[3] he states that he is undecided regarding the identity of *E. histolytica* and *E. tetragena*, and thus describes the types of entamebae observed in his cases:

"In the active vegetative stage, associated with dysentery, those entamebae which exhibited the characteristics of *histolytica* did not go on to cyst formation, but persisted unchanged, even when the stools were semisolid, except for the formation, in some instances, of the so-called chromidia. Two of these cases were carefully followed for a period of three weeks. On the other hand, in all but one instance, the infections in

[1] *Jour. Trop. Med.*, 1912, 2, p. 27.

[2] *Op. cit.*

[3] *New York Med. Jour.*, 1913, 97, p. 702.

which the entamebae were of the characteristic *tetragena* type went on to cyst formation, with a marked remission, even an apparent cure of the symptoms, and without medication."

Regarding the value of nuclear structure in the differentiation of entamebae, James says:

"I do not find that nuclear morphology, in specimens from stools with pus and blood, is a definite factor in the determination of species. Even with the most careful differentiation, many of the organisms in undoubted *tetragena* infections show nuclei that are exactly similar to those described for *histolytica;* and more than once, in infections in which the entamebae were nearly all of the *histolytica* type, and when there was no cyst formation, typical *tetragena* nuclei were observed. . . . . Also, I have not, at any time, been able to observe the 'spore formation' described by Schaudinn and others."

The literature reviewed presents the present opinions regarding the identity of *E. histolytica* and *E. tetragena*, and shows very clearly, I think, that every investigator mentioned has really been studying the same parasite but variously interpreting certain authorities upon appearances noted during different stages in its life history. A careful perusal of the literature leads one almost inevitably to the conclusion that we have really been working with only one species of entameba, but our imperfect understanding of the whole life cycle, and the unwarranted emphasis laid by certain authorities upon nuclear structure as a means of differentiating species, has prevented the recognition of the identity of these parasites by many observers.

PERSONAL OBSERVATIONS.

At the time of the appearance of Schaudinn's paper upon the entamebae, I was so fortunate as to be in charge of the laboratories of the U.S. Army General Hospital at San Francisco where hundreds of patients suffering from entamebic dysentery were being received from the Philippines, and the material for the study of entamebae was almost unlimited. As a result of the study of these parasites I published in 1905[1] a paper confirming Schaudinn's findings, and this paper was followed by another in 1908[2] amplifying and confirming the observations already reported. In these papers I recognized the existence of *E. coli*, the harmless entameba, and *E. histolytica*, the pathogenic species, and confirmed Schaudinn's description of reproduction by "budding" or spore formation in the latter species.

[1] *Op. cit.* [2] *Jour. Infect. Dis.*, 1908, 5, p. 324.

In 1911 I reported the occurrence of *E. tetragena* in the Philippine Islands, Panama, and the United States,[1] and recognized this organism as a distinct species, basing this conclusion upon the occurrence of cysts containing four nuclei during one stage in the life history of the parasite. At this time I stated that I had undoubtedly overlooked this species in the material examined at San Francisco, confusing it with both *E. coli* and *E. histolytica*. In other contributions, published in 1911[2] and 1912,[3] I stated my belief that there are three species of entamebae parasitic in man., i.e., *E. coli*, *E. histolytica*, and *E. tetragena*, and in a paper read before the Fifteenth International Congress for Hygiene and Demography, in September, 1912,[4] I stated my position regarding the identity of *histolytica* and *tetragena* as follows:

"At the present time some authorities believe that this species [*histolytica*] is identical with *Entameba tetragena*, but the point is far from proved, and until it can be shown that the process of reproduction by gemmation and spore formation, described by Schaudinn as characteristic of *Entameba histolytica*, and confirmed by myself in many cases of dysentery, occurs also in *Entameba tetragena*, and that cases of dysentery occur which never present the characteristic four nucleated cyst at any stage of the disease process, in infections with *tetragena*, I am forced to consider the two species as distinct, despite the fact that the nuclear structure of *Entameba tetragena*, during certain stages of development, resembles very closely that of *Entameba histolytica*."

As the result of the study of material since the above paper was written I have become convinced that these two so-called species are really identical, and that the process of reproduction by spore formation, described by Schaudinn, and confirmed by myself and others, is actually a degenerative change occurring especially in cases under treatment. Furthermore, I am satisfied that in this class of cases, and in cases presenting active symptoms of dysentery, *E. histolytica* does not undergo encystment, in the vast majority of instances, but reproduces only by simple division, thus explaining the absence of cysts in most of the material that I have studied.

The character of the cases studied at San Francisco must be considered in any discussion of the species of entamebae associated with them. All patients observed there had been returned to the

[1] *Arch. Int. Med.*, 1911, 7, p. 362.

[2] *The Parasitic Amoebae of Man*, Philadelphia, 1911, p. 9.

[3] *Jour. Med. Research*, 1912, 26, p. 1.

[4] *Am. Jour. Med. Sc.*, 1913, 145, p. 83.

United States from the Philippines because of the severity and intractable nature of the entamebic infections from which they were suffering; all were under treatment by means of rectal injections of quinine and other substances destructive to the entamebae; and nearly all presented active symptoms of the infection. Despite daily treatment a considerable proportion of these infections terminated fatally, and those who apparently recovered frequently relapsed, and in many the relapse proved fatal. In this class of patients the entamebae did not go on to cyst formation, and a certain, though small proportion, showed the so-called budding forms of Schaudinn. In those cases that eventually recovered, cysts were sometimes observed but were interpreted at that time as the cysts of *E. coli*, for it should be remembered that *E. tetragena* had not then been described. As the entamebae associated with these cases corresponded to Schaudinn's description of *E. histolytica*, I had no hesitation in confirming his description. As later researches have shown that in such cases cysts are seldom observed in *tetragena* infections, and as Darling has recently shown that, in infections in kittens, where the entamebae have multiplied rapidly, forms are observed similar to those described by Schaudinn, as well as the absence of cysts, I feel sure that I misinterpreted degenerative changes for a reproductive process. The fact remains, however, that too little stress has been laid upon the occurrence, in just this class of cases, of entamebae presenting the typical nuclear structure of *E. histolytica*, for most of the writers upon *tetragena* have insisted that the nuclear structure was entirely distinct from that of *histolytica*. As a matter of fact, *histolytica* presents all variation in nuclear structure from that described as typical of it, to that described as typical of *tetragena*.

The material that I have recently studied consists of three cases of entamebic dysentery occurring in the Canal Zone, preparations from which Dr. W. M. James has kindly sent me, and one case at the Soldiers' Home in Washington, whose infection was contracted in the Philippine Islands. I am greatly indebted to Dr. James for sending me specimens from his cases, for the many preparations he forwarded contain examples of nearly every stage in the life history of *E. histolytica*. The following short history of each case is

abstracted from his description of the cases which accompanied the specimens:

Case 1.—Patient a Greek, aged 49 years. Two and one-half years in the Canal Zone. Admitted January 24, 1913, with fever and diarrhea. Blood showed tertian malarial plasmodia. Stool examination on January 25 showed numerous entamebae with very faintly visible nuclei, the nuclear membrane represented by a few granules of chromatin. In a few a centriole is seen, but there is no indication of a karyosome. Ectoplasm and endoplasm easily differentiated. Stained specimens showed the typical *histolytica* type of nucleus. The entamebae remained of this type until January 30, 1913, the patient being in bed during this time upon a milk diet. On January 30, the entamebae were decidedly smaller in size, the ectoplasm less marked, and the nuclei obscured. On January 30, the nuclei were still of the *histolytica* type, but upon February 2 the entamebae were much smaller, resembling *E. minuta*, and well defined nuclei were seen. The patient had meanwhile been placed upon a diet of eggs, potatoes, toast, etc. Quite a few four-nucleated cysts were found at this date. On February 3, the stools were semi-formed and cysts were numerous, sometimes three or four in a single field. The patient was then placed on the bismuth treatment and the entamebae all disappeared from the stools in two days.

Case 2.—Patient a Spaniard. Six years in the Canal Zone. Attacks of dysentery for two months previous to admission. Admitted January 25, 1913. Stool first examined January 26, 1913. Consistence semi-fluid. Blood and mucus present. Many entamebae. In this specimen "budding" was observed in many of the entamebae, which showed nuclei of the *histolytica* type. James states that he regards the "budding" as degenerative, for the stool was over two hours old and cold at the time of examination. On January 27, the stool again contained entamebae of the *histolytica* type. The patient was placed upon soft diet and from January 31 to February 5 the stools contained smaller entamebae and numerous typical *tetragena* cysts. (My own examination of these specimens showed that entameba of both the *histolytica* and *tetragena* type were present.)

Case 3.—A French boy, aged 19 years. History of dysentery for eight days. His stools contained pus and blood. A few entamebae were seen in the vegetative stage resembling *E. minuta*. (I found besides these a few organisms of the typical *histolytica* type.) On February 9, a few cysts were observed of the *tetragena* variety.

Regarding these cases Dr. James says:

"They [the preparations] are of particular interest in that they show what I take to be *histolytica* going on to encystment. This is either true, or there is a question of a double infection with disappearance of the *histolytica* forms. This latter, however, is rather improbable when one considers that the same process happened in three consecutive cases within a few days. Heretofore I have kept my patients under observation on liquid diet, but this time all three were put on soft diet, and in each instance encystment followed within a few days."

James calls attention to the fact that the entameba found in cases showing acute symptoms is almost invariably of the *histolytica* type, while the *tetragena* type is found in more chronic cases and in infections in cats. This agrees with my own observations and dis-

proves Hartmann's statement that entamebae showing the *histolytica* type of nucleus are degenerative forms. Anyone who has studied the entamebae occurring in acute attacks of dysentery knows that they almost invariably present nuclei of the *histolytica* type, and that the *tetragena* type of nucleus occurs most often in chronic infections and just before encystment. It is evident that both Viereck and Hartmann have studied chronic cases of dysentery or they would have recognized this fact.

The following case, occurring at the Soldiers' Home in Washington, has been under my observation for three years and is of interest because it demonstrates that *E. histolytica* undergoes encystment, and that such encystment may be delayed for a long period of time.

Case 4.—An American, aged about 36. Contracted dysentery in the Philippines in 1903. First examined in February, 1909, when he was having a relapse and very active symptoms of dysentery. At this time the stools contained many entamebae of the *histolytica* type and no cysts were observed, although the stools were examined repeatedly, even after apparent recovery occurred. Since that time the man has several times suffered from a severe relapse and always entamebae of the *histolytica* type were observed in the stools during the period of active symptoms. No cysts were observed until March, 1913, when, after the absence of symptoms for several months, and treatment with ipecac and quinine irrigations had been stopped for some time, an examination of the stools, after a dose of magnesium sulfate, showed numerous typical four-nucleated cysts, and entamebae of the *histolytica* and *tetragena* types, as well as "budding" forms. As a double infection could be practically eliminated in this case the evidence is almost conclusive that *E. tetragena* is identical with *E. histolytica* and, taken in conjunction with the cases of James, is conclusive to my mind.

In the vast amount of material I have had the opportunity of studying, covering over 1,000 cases of entamebic dysentery, the following groups of cases, as regards the character of the entamebae present, have been noted:

Group 1.—Cases in which nearly all the entamebae presented the typical *histolytica* nucleus, and that never went on to cyst formation, but not infrequently showed the "budding" forms described by Schaudinn in cases apparently recovering. These cases comprised the vast majority of those studied at San Francisco and it was upon this type of entameba that I based my confirmation of Schaudinn's observations. These cases presented marked symptoms and many of them terminated fatally. Practically all were under treatment at the time of observation.

Group 2.—Cases in which the entamebae presented both the *histolytica* and *tetragena* types of nucleus and a few cysts could be found after a long search. The entamebae presenting the *tetragena* type of nucleus varied greatly in size, and the cysts contained four daughter nuclei. Until recently I had interpreted these cysts as developmental forms of the cysts of *E. coli* when they occurred in association with vegetative forms of the *histolytica* type. "Budding" forms were sometimes observed and clinically the cases were of much milder type than those in Group 1.

Group 3.—Cases in which the entamebae present were of both the *histolytica* and *tetragena* types but in which the four-nucleated cysts were present in great numbers. Clinically these cases present few or no symptoms of dysentery; the stools were semi-formed, and no treatment was being administered.

Group 4.—Cases in which all the entamebae present were smaller in size than those observed in the other groups, presented most frequently the *tetragena* type of nucleus, or a type intermediate between that and the *histolytica* type, in that, while no large karyosome was present, the nuclear membrane was much thicker than in the large vegetative forms of *histolytica*, and large numbers of four-nucleated cysts were observed. Clinically these cases presented slight or no symptoms and were not receiving treatment.

Group 5.—Cases in which there were mixed infections with *E. coli*, and entamebae answering the description of *E. histolytica* or *E. tetragena*. Clinically these cases varied greatly, as would be expected.

An analysis of the findings in these various groups of patients leads inevitably to the following conclusions:

1. Entamebae presenting the *histolytica* type of nucleus are found most frequently in patients presenting the most severe symptoms of entamebic dysentery. This type of nucleus appears to be characteristic of *E. histolytica* when it is undergoing rapid multiplication by simple division, for no cysts are observed in these cases. That this type of nucleus is not degenerative, as claimed by Hartmann, is proven by the fact that such entamebae occur most frequently in the worst cases of acute dysentery, and even though treatment is being administered, the entamebae showing this type of

nucleus differ entirely in appearance from those showing real degenerative changes brought about by the treatment. In addition, typical dividing forms are generally observed, showing this same type of nucleus.

2. Entamebae presenting the typical *tetragena* type of nucleus occur most frequently in cases presenting slight dysenteric symptoms and this type of nucleus appears to be characteristic of *E. histolytica* when it is undergoing simple division preparatory to the formation of the small generation which produces cysts.

3. Entamebae reduced in size, and presenting generally the *tetragena* type of nucleus, although an intermediate type between this and *histolytica* is frequently observed, occur most frequently in cases of long duration (chronic dysentery), in which the symptoms are practically subsiding. This type is apparently characteristic of *E. histolytica* preparatory to the formation of cysts.

4. The four-nucleated cysts occur most frequently in cases which have apparently recovered and in which the stools are semi-formed or formed. These cysts are characteristic of *E. histolytica* but occur only in a very small proportion of cases, as shown by Wenyon,[1] Ornstein,[2] James,[3] and others, and do not appear in acute infections, as shown by their absence in such infections in man and in fatal infections in kittens.

5. Entamebae presenting appearances that have been interpreted as reproduction by "budding" or spore formation occur most frequently in cases presenting subacute symptoms of dysentery. Such forms are associated with entamebae showing the *histolytica* type of nucleus and occur in cases in which the entamebae are undergoing rapid multiplication by simple division. Most of the patients were receiving treatment by rectal injections of quinine. Similar forms, much smaller in size, are also found in association with cysts and are not infrequently observed in patients who have apparently recovered. I believe that these forms are degenerative in nature and may be produced by exhaustion of the race due to rapid multiplication, as suggested by Darling, or by the effect of remedial agents, as quinine. Personally, I am of the opinion that both

[1] *Op. cit.*

[2] *Arch. f. Protistenk.*, 1913, 29, p. 78.

[3] Personal communications.

causes may operate in producing these so-called "budding" forms of *E. histolytica*.

OBSERVATIONS UPON THE MORPHOLOGY AND LIFE CYCLE OF *E. histolytica*.

In the light of our present knowledge it becomes necessary to reconstruct the life history of *E. histolytica* and to recognize that the nuclear structure of this species may vary, at different times in the life history, from that typical of *histolytica*, as described by Schaudinn, to that typical of *tetragena*, as described by Viereck and Hartmann. This fact was first clearly stated by Walker but the insistence of Hartmann and his followers upon the absolutely diagnostic structure of the nucleus in *E. tetragena* led to great confusion and delay in the recognition of the identity of this species with *E. histolytica*. Having been led astray myself because of relying too much upon morphology in the interpretation of certain phenomena occurring in the entamebae, I am convinced that too much stress has been laid upon morphological details in the differentiation of species and too little upon the study of the entire life cycle of these organisms. I agree thoroughly with James in his statement that nuclear morphology *alone* is not sufficient for the determination of species among the entamebae, for even in the hands of the most expert this method of determining species has failed not infrequently. While nuclear morphology is sufficient to differentiate the entamebae from the free living amebae of the genus *Ameba*, it is not sufficient for the determination of species within the genus *Entameba*. The nucleus of these parasites is constantly changing in morphology in answer to metabolic changes and differences in the environment. In addition, our fixing and staining methods are still far from perfect and undoubtedly produce more or less change in the appearance of the nucleus, as can be easily proven by varying the time of staining and amount of differentiating with the various solutions employed for this purpose. I am now referring to the most approved methods of wet-fixation and staining, for all of the observations recorded in this paper have been made upon wet-fixed and stained preparations.

From our present knowledge of the subject I believe that the

following description of the morphology and life history of *E. histolytica* is approximately correct. Briefly stated, the life history may be divided into the following stages: a vegetative stage of development, in which multiplication occurs rapidly by simple division: a pre-cystic stage in which multiplication occurs by simple division and the entamebae become markedly reduced in size; and a cystic stage, during which cysts containing four nuclei are developed. Of course, organisms are frequently encountered in any one of these stages, with the exception of the first, which belong to one of the other stages of development, but in the vast majority of our cases these definite stages may be observed if the conditions are favorable and the cases are followed for a long enough time. Cases that are acute or upon vigorous treatment will not show cysts, but if they become chronic and treatment is omitted, the pre-cystic and cystic stages of development can be demonstrated.

Vegetative stage of development.—It is in this stage of development that *E. histolytica* attains its largest size, sometimes exceeding 65 microns in its longest diameter, but averaging generally from 25 to 40 microns. In the living specimen there is a well marked distinction between the ecto- and endoplasm and the organism is actively ameboid. The nucleus may or may not be visible, but in most instances is invisible in acute cases of dysentery. As two distinct types of nuclei may occur during this stage of development, it is only when organisms presenting the *tetragena* type of nucleus are present that we are able to distinguish a nucleus in the living specimen. The cytoplasm contains bacteria, crystals, and in cases in which blood is present in the feces, one or more erythrocytes. Vacuoles are present but are not contractile.

In preparations wet-fixed and stained with iron hematoxylin or other hematoxylin stains, there is no distinction between the ecto- and endoplasm, the cytoplasm appearing coarsely granular or containing numerous small vacuoles or one or more large vacuoles. Two distinct types of nucleus are present in these vegetative entamebae, the so-called *histolytica* type and the *tetragena* type. In the former type the nuclear membrane is well stained and consists of a delicate, very thin membrane, upon the inner side of which a few minute chromatin granules may be observed. This membrane

often appears not over a line in thickness. A very small and delicate karyosome, called by some authors a centriole, often appearing simply as a minute dot of chromatin, is generally observed at or near the center of the nucleus. A centriole is not present within this karyosome.

A few minute dots or grains of chromatin may sometimes be seen lying within the nucleus between the karyosome and the nuclear membrane and traces of a linin network may sometimes be observed. The entire nucleus appears delicate and is often situated at the extreme periphery of the organism.

Reproduction by simple division is frequently observed in entamebae showing this type of nucleus, and in almost every specimen examples of this type of reproduction may be observed. In most instances the two nuclei produced by division are exactly similar in structure, and that structure is of the *histolytica* type, but rarely an entameba is seen containing one nucleus of the *histolytica* type and one of the *tetragena* type. Such an organism is illustrated in Fig. 6 and definitely proves that both types of nuclei are present in *E. histolytica.*

This type of nucleus is most frequently observed, as has been stated, in acute attacks of dysentery and is typical of most of the entamebae seen in the feces when severe symptoms are present. In cases showing less marked symptoms, or where the disease has become chronic, the vegetative entamebae generally show nuclei of the *tetragena* type. In this type the nuclear membrane is much thicker and better defined, while there is a comparatively large karyosome, situated at or near the center of the entameba, which contains a well marked centriole. The chromatin within the nucleus is much larger in amount than in the *histolytica* type, and is either scattered upon the inner side of the nuclear membrane and as minute, deeply stained masses upon the linin network between the membrane and the karyosome, or as larger, irregularly shaped masses upon the inner surface of the nuclear membrane. The karyosome is characteristic, consisting of a large, irregular or spherical mass of chromatin, often appearing connected with the nuclear membrane by delicate filaments. A centriole is often present consisting of a deeply stained, spherical dot of chromatin surrounded by a well

marked, unstained halo. Many variations are observed in the structure of this type of nucleus which are believed by Hartmann to by cyclical in character. The karyosome sometimes appears as a fine network inclosing an unstained area within which lies the centriole, while the outer border of the karyosome is formed of deeply staining dots of chromatin arranged in a circular manner. Again, the karyosome may stain evenly throughout with the exception of a clear, unstained halo around the centriole, or the halo may be absent, the centriole appearing as a deeply staining, almost black, mass within the karyosome. Rarely the entire karyosome is surrounded by an unstained area, the remainder of the nucleus presenting a dimly stained network having arranged upon it deeply stained dots of chromatin, or the chromatin may be arranged in bandlike masses near the nuclear membrane.

In many cases, if one searches carefully, intermediate types of nuclei between the *histolytica* and *tetragena* types may be seen, and a whole series of nuclei may be traced from that typical of *histolytica*, as described by Schaudinn, to that typical of the *tetragena* nucleus, described by Viereck and Hartmann.

In subacute cases of entamebic dysentery the so-called "budding" forms of *E. histolytica* may be found associated with these vegetative stages in the life history of the organism. These forms are most often found after the acute symptoms have lasted for some time and are just beginning to decline, generally as the result of treatment with rectal injections of various therapeutic agents. In other words, they are most often encountered when the race of entamebae has become weakened by rapid multiplication and by therapeutic measures. Organisms are observed in which the chromatin is being ejected from the nucleus and distributed in the cytoplasm. Others are noted in which the nucleus has disappeared and the entire cytoplasm is filled with masses of chromatin, while still others are observed in which these masses of chromatin are being "budded" off from the periphery of the parasite, surrounded by a small amount of cytoplasm. Many of these "buds" appear to develop a membrane around them and no structure can be distinguished. I am now convinced that all of these forms are degenerative in character and have nothing to do with any process of reproduction.

Pre-cystic stage of development.—In this stage of development the entamebae multiply by simple division, but become markedly reduced in size, resembling the entameba described by Elmassian as *E. minuta*. The majority of the organisms also show a nucleus which may be said to be intermediate in structure between that of the *histolytica* and *tetragena*, having a thick nuclear membrane and a small, solid appearing karyosome. While entamebae are found at this stage with nuclei like those of both the *histolytica* and *tetragena* types, most of the organisms show nuclei like that described. The most marked feature at this stage of development is the small size of the vegetative forms, and to this generation of the parasite, James and Darling have given the name "the small pre-cystic generation."

Most cases showing these forms will also show a few typical four-nucleated cysts if they are carefully searched for, although there may not be more than one or two found in a single preparation.

Cystic stage of development.—The cystic stage of development quickly follows the pre-cystic. In cases showing cysts the vegetative forms, just described as characteristic of the pre-cystic stage, may be present in very small numbers, but when cyst formation is complete these vegetative forms are generally absent.

The organisms which become encysted generally contain more or less chromatin in their cytoplasm and in both the pre-cystic and cystic stages of development forms are rarely observed which resemble the "budding" forms found most frequently in the vegetative stage but they are much smaller and are really degenerated pre-cystic or cystic forms.

When the cyst is fully developed, the chromidia will be found collected in one or more spindle-shaped masses within the cyst, which stain deeply and can readily be distinguished from any nuclei that may be present. The cysts measure from 7 to 20 microns in diameter, the average being about 15 microns, and are spherical in shape. The smaller cysts exactly resemble those described as characteristic of *E. minuta*. A distinct membrane may rarely be observed surrounding the cyst in stained preparations. The cytoplasm stains uniformly, and vacuoles are absent unless the cyst is degenerating, while extraneous matter, such as bacteria, erythro-

cytes, etc., has been extruded before the formation of the cystic membrane. The nucleus prior to division resembles that found in the vegetative form in the pre-cystic stage, having a rather thick nuclear membrane and a small, but distinct karyosome (centriole, of some writers). The nucleus divides into two nuclei, reduced somewhat in size, which have a very definite nuclear membrane and a well defined karyosome, composed of a few minute granules or a solid mass of chromatin. These two nuclei divide, the result being a cyst containing four nuclei. At the final stage of division the nuclei are much reduced in size and each of the four possesses a well defined nuclear membrane, thin in outline, and a minute karyosome arranged as a solid mass of chromatin somewhat irregular in shape, or as a minute collection of chromatin granules.

Many of the four-nucleated cysts do not show the large spindle-shaped masses of chromatin alluded to above, so that it is evident that these have nothing to do with the vital processes involved in the formation of the four nuclei.

This brief description of the forms of *E. histolytica* observed in the various stages of its life history covers very inadequately the many variations that occur in every stage, but gives as composite a picture of the morphology of this interesting and important parasite as we are able to give at present. It has been proven that the cystic stage is alone infective to cats, and thus it is evident that in human infections the patients who show cysts are the ones that are a menace to others. As pointed out by Walker, these patients are really "carriers" of entamebic dysentery and the greatest care should be taken in the disposal of their excreta.

Nomenclature and classification.—I believe that it is best, at the present time, to recognize but two species of entamebae as being parasitic in the intestine of man, *E. coli* and *E. histolytica*. I think that the researches of several investigators have proven that *E. tetragena* is identical with *E. histolytica*, and the other species that have been described have not been confirmed and are still of uncertain value.

If this classification be adopted, the name *tetragena* should be dropped, as it becomes merely a synonym of *histolytica*. The fact that Schaudinn incorrectly described a portion of the life history of

histolytica and overlooked the cystic stage of development does not vitiate the status of this parasite as a distinct species. All authorities are practically agreed upon the fact that Schaudinn differentiated a harmless from a pathogenic species of entameba, and it makes no difference, so far as the species are concerned, whether his entire description was correct or not. He did describe correctly the vegetative stage of the parasite as observed in the vast majority of cases of dysentery presenting severe symptoms, and distinguished this species from *E. coli*, the species he found in healthy individuals. Therefore the name *Entameba histolytica*, which he gave this pathogenic species, must, by the law of priority, be retained as the specific name, and the name *tetragena* ceases to have any specific value. This conception of the specific name depends, of course, upon the accuracy of the name Schaudinn employed. If, as some believe, the pathogenic Entameba should have been named *Entameba coli* (Lösch) or *Entameba dysenteriae* (Councilman and Lafleur), either of these names should replace *histolytica*. At any rate, the name *tetragena* has now no status as a specific name.

CONCLUSION.

In concluding the discussion of these entamebae I cannot refrain from calling attention to the misinterpretations which are rendered almost unavoidable when protozoölogists insist upon laying so much stress upon minute morphological details in so variable a structure as the nucleus, in the differentiation of species of entamebae. Hartmann's imperfect description of *E. histolytica* (*E. tetragena*) has confused almost every worker on these organisms, as his omission of the fact that the typical *histolytica* nucleus occurs in the most acute cases of dysentery, while the *tetragena* type of nucleus is found in milder cases, and his really absurd statement that the *histolytica* type of nucleus is a degenerative form has, of course, resulted in most authorities regarding *histolytica* and *tetragena* either as distinct species or that Schaudinn's description of *histolytica* was entirely erroneous.

In a recent communication received from Dr. W. M. James, of Panama, he agrees with me that *E. histolytica* and *E. tetragena* are identical and that the *histolytica* type of nucleus is found chiefly in acute and severe infections. His observations will be published later, but they confirm, in almost every detail, all that is published in this paper.

I desire to take this opportunity of acknowledging my indebtedness to Dr. James for the many excellent preparations he has sent me and for a great deal of valuable data upon the entamebae he has studied in the Canal Zone.

EXPLANATION OF PLATES 1 AND 2.

The photomicrographs[1] were taken with a No. 2 eyepiece and a one-twelfth inch immersion objective. All specimens were wet-fixed and stained with iron hematoxylin.

FIG. 1.—Vegetative form (trophozoite) of *E. histolytica*, showing typical *histolytica* type of nucleus. ×1300.

FIG. 2.—Vegetative form of *E. histolytica*, showing a modified *tetragena* type of nucleus. ×1300.

FIG. 3.—Vegetative forms of *E. histolytica*, showing *tetragena* type of nucleus. The karyosome is too deeply stained to show the centriole. ×1300.

FIG. 4.—Vegetative form of *E. histolytica* undergoing simple division. The nucleus has just divided and the two new nuclei are of the *tetragena* type. ×1300.

FIG. 5.—Vegetative form of *E. histolytica* undergoing simple division. The two new nuclei have separated and are of a modified *tetragena* type. ×1300.

FIG. 6.—Vegetative form of *E. histolytica* undergoing simple division. This organism is of great interest as it shows one nucleus having a typical *histolytica* structure and one having just as typical a *tetragena* structure. ×1300.

FIG. 7.—A so-called "budding" form of *E. histolytica*. This form has been misinterpreted as multiplication by spore formation. The chromatin is scattered throughout the cytoplasm and some of it is being budded off from the periphery of the parasite. ×1300.

FIG. 8.—Pre-cystic vegetative form of *E. histolytica*. Note reduced size and intermediate type of nucleus, the karyosome being larger than in the large vegetative *histolytica* and smaller than in the *tetragena* type of nucleus. ×1200.

FIG. 9.—*E. histolytica* just at the time of encystment, containing one nucleus similar in structure to that in Fig. 8. ×1150.

FIG. 10.—Encysted form of *E. histolytica*, showing the primary division of the nucleus into two daughter nuclei. ×1150.

FIG. 11.—Encysted form of *E. histolytica*, showing three daughter nuclei. The fourth nucleus, if ever present, has degenerated and is not visible. ×1150.

FIG. 12.—Encysted form of *E. histolytica*, showing four daughter nuclei. This is the fully developed form of the cyst and is typical of this species. ×1150.

FIGS. 13 AND 14.—Degenerated cystic forms of *E. histolytica*, showing the distribution of the chromatin in masses in the cystoplasm. These forms have also been misinterpreted as "budding" forms or reproduction by spore formation, by some authorities. ×1150.

[1] I am indebted to Captain Arthur R. Christie for the photomicrographs here reproduced.

PLATE 1.

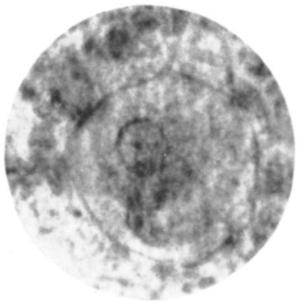


FIG. 1.

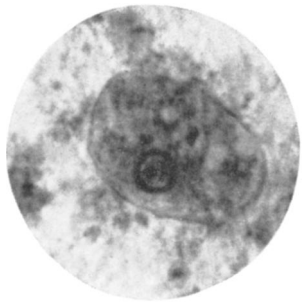
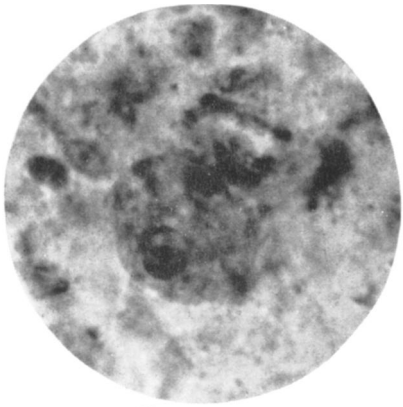


FIG. 2.



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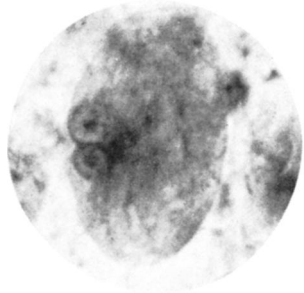

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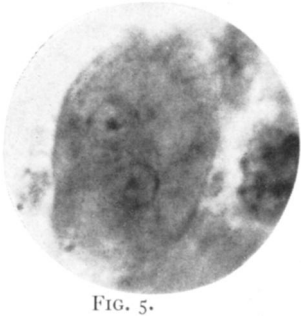

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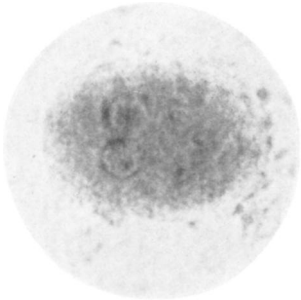

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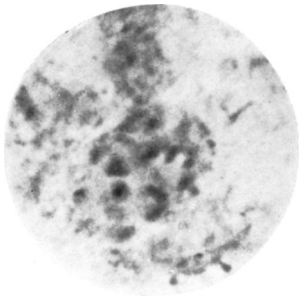

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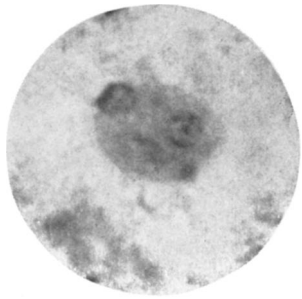


FIG. 8.

PLATE 2.

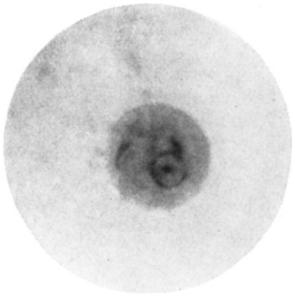
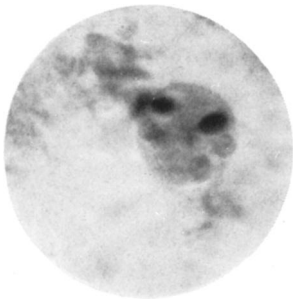


FIG. 9.

FIG. 10.



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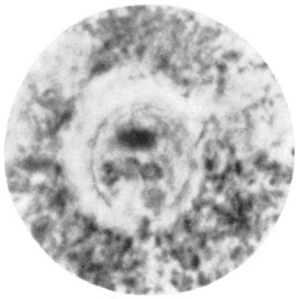

FIG. 12.

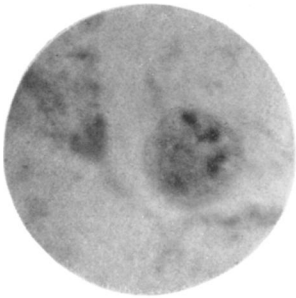

FIG. 13.

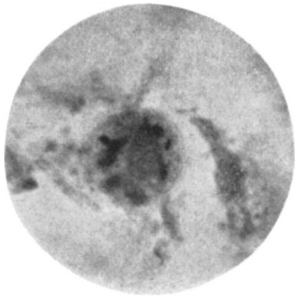

FIG. 14.

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The photomicrographs[1] were taken with a No. 2 eyepiece and a one-twelfth inch immersion objective. All specimens were wet-fixed and stained with iron hematoxylin.

FIG. 1.—Vegetative form (trophozoite) of *E. histolytica*, showing typical *histolytica* type of nucleus. ×1300.

FIG. 2.—Vegetative form of *E. histolytica*, showing a modified *tetragena* type of nucleus. ×1300.

FIG. 3.—Vegetative forms of *E. histolytica*, showing *tetragena* type of nucleus. The karyosome is too deeply stained to show the centriole. ×1300.

FIG. 4.—Vegetative form of *E. histolytica* undergoing simple division. The nucleus has just divided and the two new nuclei are of the *tetragena* type. ×1300.

FIG. 5.—Vegetative form of *E. histolytica* undergoing simple division. The two new nuclei have separated and are of a modified *tetragena* type. ×1300.

FIG. 6.—Vegetative form of *E. histolytica* undergoing simple division. This organism is of great interest as it shows one nucleus having a typical *histolytica* structure and one having just as typical a *tetragena* structure. ×1300.

FIG. 7.—A so-called "budding" form of *E. histolytica*. This form has been misinterpreted as multiplication by spore formation. The chromatin is scattered throughout the cytoplasm and some of it is being budded off from the periphery of the parasite. ×1300.

FIG. 8.—Pre-cystic vegetative form of *E. histolytica*. Note reduced size and intermediate type of nucleus, the karyosome being larger than in the large vegetative *histolytica* and smaller than in the *tetragena* type of nucleus. ×1200.

FIG. 9.—*E. histolytica* just at the time of encystment, containing one nucleus similar in structure to that in Fig. 8. ×1150.

FIG. 10.—Encysted form of *E. histolytica*, showing the primary division of the nucleus into two daughter nuclei. ×1150.

FIG. 11.—Encysted form of *E. histolytica*, showing three daughter nuclei. The fourth nucleus, if ever present, has degenerated and is not visible. ×1150.

FIG. 12.—Encysted form of *E. histolytica*, showing four daughter nuclei. This is the fully developed form of the cyst and is typical of this species. ×1150.

FIGS. 13 AND 14.—Degenerated cystic forms of *E. histolytica*, showing the distribution of the chromatin in masses in the cystoplasm. These forms have also been misinterpreted as "budding" forms or reproduction by spore formation, by some authorities. ×1150.

[1] I am indebted to Captain Arthur R. Christie for the photomicrographs here reproduced.